

[CLAIMS]

1) A clean synthetic vector containing only the elements indispensable to its functionality and to the transgenesis of a cell, said vector comprising, as operationally bound elements indispensable to its functionality and to the transgenesis of a cell:

5           - a nucleic acid sequence coding for a first origin of replication;

          - a nucleic acid sequence coding for a selection agent; and

          - a trfA locus coding for a protein that permits an increase in the replication rate of the vector.

2. The clean synthetic vector of claim 1 wherein said cell is a plant cell.

3. The clean synthetic vector of claim 1 wherein said first origin of replication is an RK2 ori.

4. The clean synthetic vector of claim 3 wherein said RK2 ori is a V ori with a broad host range.

5. The clean synthetic vector of claim 1 wherein said selection agent is an antibiotic resistance gene.

6. The clean synthetic vector of claim 5 wherein said antibiotic resistance gene is an npt III gene that confers resistance to kanamycin in bacteria.

7. The clean synthetic vector of claim 1 wherein said trf locus coding for a protein that permits an increase in the replication rate of the vector originates from pRK2.

20           8. The clean synthetic vector of claim 7 wherein said locus coding for a protein that permits an increase in the replication rate of the vector encodes P285 and P382.

9) The clean synthetic vector of claim 1 wherein said vector comprises the nucleic acid sequence identified by the number SEQ.ID01.

10) The clean synthetic vector of claim 1 wherein said vector is pMRT1105, whose nucleic acid sequence is identified by the number SEQ.ID01.

11) The clean synthetic vector of claim 1 wherein said vector comprises a nucleic acid sequence coding for a second origin of replication

5 12. The clean synthetic vector of claim 11 wherein said second origin of replication is an *E. coli* ori.

13. The clean synthetic vector of claim 12 wherein said *E. coli* ori is a *ColE1* ori.

14) The clean synthetic vector of claim 1 wherein said vector comprises the nucleic acid sequence identified by the number SEQ.ID02.

15) The clean synthetic vector of claim 1 wherein said vector is plasmid pMRT1106, whose nucleic acid sequence is identified by the number SEQ.ID02.

16) The clean synthetic vector of claim 1 wherein said vector comprises a region comprising a nucleic acid sequence containing a plurality of unique enzyme restriction sites, said plurality collectively called a «multiple cloning site» (MCS).

17) The clean synthetic vector of claim 1 wherein said vector comprises a nucleic acid sequence coding for a T-DNA, including a right border, RB, and a left border, LB, which permit the vector to function as a binary plasmid.

18) The clean synthetic vector of claim 16 or claim 17 wherein said MCS is situated near the right border RB of the T-DNA.

20 19) The clean synthetic vector of claim 17 wherein said vector comprises a nucleic acid sequence coding for at least one expression promoter and at least one transcription terminator situated between the left border, LB, and the right border, RB, of the T-DNA.

20 20) The clean synthetic vector of claim 19, wherein said expression promoter is chosen from the group consisting of constitutive promoters, inducible promoters and specific promoters.

21) The clean synthetic vector of claim 19 wherein said expression promoter is a plant expression promoter.

22) The clean synthetic vector of claim 21 wherein said expression promoter is chosen from the group consisting of: the 35S CaMV promoter; the ep35S of CaMV; the pea plastocyanin gene promoter, its “enhancer” and derived zones; the “high molecular weight glutenin” (HMWG) promoter of wheat; the CsVMV “Cassava mosaic virus” promoter; the CoYMV “Commelina yellow mosaic virus” promoter; the chimeric promoters of the CsVMV and CoYMV promoters; and derivatives thereof.

23) The clean synthetic vector of claim 19 wherein said expression terminator is chosen from the functional terminators in a plant cell.

24) The clean synthetic vector of claim 23 wherein said functional terminator is a 35S or a nos terminator.

25) The clean synthetic vector of claim 1 wherein said vector comprises a nucleic acid sequence coding for a selection agent that is functional in a plant cell.

26) The clean synthetic vector of claim 25 wherein said nucleic acid sequence coding for a selection agent codes for an antibiotic resistance gene and/or an herbicide resistance gene.

27) The clean synthetic vector of claim 26, wherein said vector comprises a sequence coding for the bar resistance («bialaphos resistance») or pat («phosphinothricin acetyltransferase») gene.

28) The clean synthetic vector of claim 25, wherein said nucleic acid sequence coding for a selection agent that is functional in a plant cell is a sequence coding for a mutant or wild-type nptII resistance gene.

29) The clean synthetic vector of claim 25 wherein said nucleic acid sequence coding for a selection agent is situated near the left border of said T-DNA.

30) The clean synthetic vector of claim 1 wherein said vector comprises an expression cassette comprising an expression-promoting nucleic acid sequence operationally bound to a nucleic acid sequence encoding a polypeptide to be expressed, wherein said nucleic acid sequence encoding a polypeptide to be expressed is itself operationally bound to a transcription 5 termination nucleic acid sequence.

31) The clean synthetic vector of claim 30, wherein said polypeptide to be expressed is an enzyme or protein or derivative thereof possessing an activity in vitro and/or in man and/or in animals, said activity selected from a digestive, pancreatic, biliary, antiviral, anti-inflammatory, 10 pulmonary, antimicrobial, nutritional, cosmetic, structural, blood, cardiovascular, ophthalmic, antigenic, immunostimulatory or cerebral activity.

32) A vector of claim 1, said vector being a binary, linear or circular plasmid.

33) A vector of claim 32, said vector chosen from the group consisting of the nucleic acid sequences identified by the numbers SEQ.ID03, SEQ.ID04, SEQ.ID05, SEQ.ID06, SEQ.ID07, SEQ.ID08, SEQ.ID09, SEQ.ID10, SEQ.ID11, SEQ.ID12, SEQ.ID13, SEQ.ID14, SEQ.ID15, SEQ.ID16, SEQ.ID17, SEQ.ID18, SEQ.ID19, SEQ.ID20, SEQ.ID21 and SEQ.ID22.

34) The clean synthetic vector of claim 1 wherein each functional component can be cleaved independently of the other components.

35) The clean synthetic vector of claim 34, wherein each functional component can be cleaved independently of the other components by enzymatic digestion at a first unique 20 restriction site and a second unique restriction site, both sites being present in one vector.

36) A nucleic acid sequence, said sequence comprising a nucleic acid sequence chosen from the group consisting of the nucleic acid sequences identified by the numbers SEQ.ID01, SEQ.ID02, SEQ.ID03, SEQ.ID04, SEQ.ID05, SEQ.ID06, SEQ.ID07, SEQ.ID08, SEQ.ID09, SEQ.ID10, SEQ.ID11, SEQ.ID12, SEQ.ID13, SEQ.ID14, SEQ.ID15, SEQ.ID16, SEQ.ID17, 25 SEQ.ID18, SEQ.ID19, SEQ.ID20, SEQ.ID21 and SEQ.ID22.

37) A transgenic plant having stably integrated in its genome a vector of claim 1.

38) A transgenic plant having stably integrated in its genome a nucleic acid sequence of claim 36.

39) The transgenic plant of claim 37 or claim 38 wherein said plant is a dicotyledon species.

5 40) The transgenic plant of claim 39 wherein said dicotyledon species is selected from the group consisting of potato, tobacco, cotton, lettuce, tomato, melon, cucumber, pea, rape, beetroot and sunflower.

41) The transgenic plant of claim 37 or claim 38 wherein said plant is a monocotyledon species.

10 42) The transgenic plant of claim 41 wherein said monocotyledon species is selected from the group consisting of wheat, barley, oats, rice and maize.

43) A propagule of a transgenic plant of claim 37 or 38.

44) The propagule of claim 43 wherein said propagule is a seed.

45) A cell comprising a vector of claim 1.

15 46) A cell comprising a nucleic acid sequence selected from the group consisting of those sequences identified by the numbers SEQ.ID01, SEQ.ID02, SEQ.ID03, SEQ.ID04, SEQ.ID05, SEQ.ID06, SEQ.ID07, SEQ.ID08, SEQ.ID09, SEQ.ID10, SEQ.ID11, SEQ.ID12, SEQ.ID13, SEQ.ID14, SEQ.ID15, SEQ.ID16, SEQ.ID17, SEQ.ID18, SEQ.ID19, SEQ.ID20, SEQ.ID21 and SEQ.ID22.

20 47) The cell of claim 45 or claim 46 wherein said cell is a plant cell.

48) A method for the expression of a nucleic acid sequence coding for a polypeptide to be produced in a cell, said method comprising the steps of:

- transforming said cell with a vector of claim 1, said vector comprising said nucleic acid sequence coding for said polypeptide; and

- maintaining said cell under conditions that permit the expression of said nucleic acid sequence coding for said polypeptide, whereby said polypeptide is produced.

49) A method for the expression of a nucleic acid sequence coding for a polypeptide to be produced in a cell, said method comprising the steps of:

5 - transforming said cell with a nucleic acid sequence of claim 36, said sequence comprising said nucleic acid sequence coding for said polypeptide; and

- maintaining said cell under conditions that permit the expression of said nucleic acid sequence coding for said polypeptide, whereby said polypeptide is produced.

50) The method of claim 48 or 49 wherein said cell is a prokaryotic or eukaryotic cell.

51) The method of claim 48 or 49 wherein said cell is chosen from the group consisting of microbial cells, fungal cells, insect cells, animal cells and plant cells.

52) The method of claim 48 or 49 wherein said cell is a plant cell.

53) A method for obtaining a transgenic plant of claim 37, said method comprising the steps of:

a) transforming a plant cell with a vector of claim 1;

b) selecting a plant cell having said vector integrated therein; and

c) propagating the selected plant cell of step (b), either by culturing or by regeneration of chimeric or transgenic whole plants.

54. A method for obtaining a transgenic plant of claim 38, said method comprising the

20 steps of:

a) transforming a plant cell with a nucleic acid of claim 36;

b) selecting a plant cell having said vector integrated therein; and

c) propagating the selected plant cell of step (b), either by culturing or by regeneration of chimeric or transgenic whole plants.